Sensing and translation of pathogen signals into demand-adapted myelopoiesis

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Abstract: PURPOSE OF REVIEW: During severe systemic infection, steady-state hematopoiesis is switched to demand-adapted myelopoiesis, leading to increased myeloid progenitor proliferation and, depending on the context and type of pathogen, enhanced granulocytic or monocytic differentiation, respectively. We will review the recent advances in understanding direct and indirect mechanisms by which different pathogen signals are detected and subsequently translated into demand-adapted myelopoiesis. RECENT FINDINGS: Enhanced myeloid progenitor proliferation and neutrophil differentiation following infection with prototypic Gram-negative bacterium Escherichia coli is mediated by granulocyte colony-stimulating factor, and reactive oxygen species released from endothelial cells and mature myeloid cells, respectively. Furthermore, hematopoietic stem and progenitor cells directly sense pathogen signals via Toll-like receptors and contribute to emergency granulopoiesis via release and subsequent autocrine and paracrine action of myelopoietic cytokines including IL-6. Moreover, emergency monocytopoiesis upon viral infection depends on T cell-derived IFN and release of IL-6 from bone marrow stromal cells. SUMMARY: A complex picture is evolving in which various hematopoietic and nonhematopoietic cell types interact with the hematopoietic system in an intricate manner to shape an appropriate hematopoietic response to specific infectious stimuli.

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Sensing and translation of pathogen signals into demand-adapted myelopoiesis

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During severe systemic infection, steady-state hematopoiesis is switched to demand-adapted myelopoiesis, leading to increased myeloid progenitor proliferation and, depending on the context and type of pathogen, enhanced granulocytic or monocytic differentiation, respectively. We will review the recent advances in understanding direct and indirect mechanisms by which different pathogen signals are detected and subsequently translated into demand-adapted myelopoiesis.

Recent findings
Enhanced myeloid progenitor proliferation and neutrophil differentiation following infection with prototypic Gram-negative bacterium *Escherichia coli* is mediated by granulocyte colony-stimulating factor, and reactive oxygen species released from endothelial cells and mature myeloid cells, respectively. Furthermore, hematopoietic stem and progenitor cells directly sense pathogen signals via Toll-like receptors and contribute to emergency granulopoiesis via release and subsequent autocrine and paracrine action of myelopoietic cytokines including IL-6. Moreover, emergency monocytopoiesis upon viral infection depends on T cell-derived IFNγ and release of IL-6 from bone marrow stromal cells.

Summary
A complex picture is evolving in which various hematopoietic and nonhematopoietic cell types interact with the hematopoietic system in an intricate manner to shape an appropriate hematopoietic response to specific infectious stimuli.

Keywords
cytokines and growth factors, granulopoiesis, hematopoietic stem and progenitor cells, infection and inflammation, monocytopoiesis

INTRODUCTION

The hematopoietic system is maintained by a rare population of predominantly quiescent but intermittently actively cycling hematopoietic stem cells (HSCs) that give rise to several lineage-restricted hematopoietic progenitor cells, ultimately yielding cells of the various mature myeloid and lymphoid lineages [1]. As most of the mature hematopoietic cells are terminally differentiated, nondividing and short-lived, the unique functional organization of the hematopoietic system needs to ensure homeostatic cell counts in peripheral blood during steady state by enormous cellular multiplication that occurs during lineage maturation [1]. However, pathologic conditions that disturb the hematopoietic equilibrium such as bleeding or severe systemic infection induce demand-adapted hematopoietic response programs that not only counterbalance cell losses but also drastically increase cellular output to meet the specific needs during emergency situations [2].

In contrast to uncomplicated bacterial and viral infections that are contained locally by innate and adaptive immune defense mechanisms, the response to severe systemic infection with classical Gram-positive or Gram-negative bacteria (e.g., *Staphylococci* or *Enterobacteriaceae*) leads to a number of well known and characteristic clinical phenomena, such as peripheral blood leukocytosis, neutrophilia, and 'left-shift' (i.e., the appearance of immature neutrophil precursor cells in the peripheral blood). These systemic signs of infection are caused by two complementary functional cascades

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KEY POINTS

- Endothelial cells translate the detection of systemic Gram-negative bacterial infection into emergency granulopoiesis via *Myd88*-dependent sensing of LPS and subsequent release of G-CSF.
- ROS are released from bone marrow-resident $Gr1^+$ mature myeloid cells and promote emergency granulopoiesis independent of G-CSF.
- Bone marrow mesenchymal stromal cells secrete IL-6 upon stimulation with IFNγ released from $CD8^+$ cytotoxic T cells, and IL-6, in turn, promotes emergency monocytopoiesis from early HSPCs during systemic viral infection. In addition, IFNγ directly acts on HSPCs favoring monocytic lineage differentiation.
- HSPCs secrete various inflammatory cytokines and growth factors following direct stimulation with TLR2 and TLR4 agonists. HSPC-derived cytokines contribute to initiation of emergency granulopoiesis albeit under very specific experimental conditions.

launched upon severe systemic bacterial infection: neutrophil recruitment, which is beyond the scope of this review but has excellently been reviewed lately [3], and 'emergency granulopoiesis'. Emergency granulopoiesis describes the processes leading to the *de novo* generation of granulocytes (most importantly neutrophils) and it is a prototypic example for demand-adapted hematopoiesis [4▪]. However, during severe infections with other types of pathogens (i.e., intracellularly replicating bacteria, viruses etc.), the dominant myelopoietic response is an enhanced production of monocytes, that is, 'emergency monocytopoiesis', sometimes paralleled by suppression of granulopoiesis. Throughout this review we will thus use the term 'demand-adapted myelopoiesis' to describe infection-induced hematopoietic responses in general, and we will utilize the specific terms 'emergency granulopoiesis' and 'emergency monocytopoiesis' to specify the primary cellular outcome.

The essential first step in the initiation of demand-adapted myelopoiesis is detection of the presence of a pathogenic organism (e.g., bacteria, virus, and fungi) to indicate the emergency condition to the hematopoietic system, followed by a cascade of molecular events that induce increased proliferation and myeloid differentiation of hematopoietic progenitor cells. Despite the obvious fundamental importance of initiation of demand-adapted myelopoiesis, its exact mechanisms have only begun to be elucidated over the last years. From a theoretical point of view, a putative cell type initiating demand-adapted myelopoiesis has to meet two requirements: it needs to have a high likelihood of encountering an invading pathogen and it has to be equipped with the molecular machinery for pathogen sensing and subsequent stimulation of myelopoiesis. Pattern recognition receptors such as Toll-like receptors (TLRs) sense pathogens through detection of highly conserved pathogen-associated molecular patterns triggering several molecular cascades critical for proper immune and hematopoietic responses to infection. In light of the broad distribution and cooccurrence of these two functional prerequisites among the various cells of the mammalian organism including hematopoietic and nonhematopoietic cells, two principal mechanisms of initiation of demand-adapted myelopoiesis, that is, indirect and direct, can be considered. A model of indirect initiation of demand-adapted myelopoiesis would incorporate cells present in the bone marrow and/or the peripheral tissue acting solely as pathogen sensors but not generating myeloid offspring themselves. In this case, demand-adapted myelopoiesis is stimulated by secondary release of myelopoietic cytokines and growth factors from the pathogen sensing cell type. By contrast, direct pathogen sensing by hematopoietic stem and progenitor cells (HSPCs) would directly induce enhanced proliferation and myeloid-biased differentiation.

In this review, we will discuss the evidence for both models with an emphasis on recent findings revealing a complex system of multiple complementary mechanisms that have evolved to ensure proper hematopoietic responses toward potentially life-threatening systemic infections.

INDIRECT INITIATION OF DEMAND-ADAPTED MYELOPOIESIS- PATHOGEN SENSING VIA HEMATOPOIETIC AND NONHEMATOPOIETIC CELLS

An abundance of studies conducted over the past decades has clearly demonstrated that steady-state myelopoiesis is driven via the well orchestrated activity of various myelopoietic growth factors [5,6]. Of these, granulocyte colony-stimulating factor (G-CSF) is the best studied cytokine [7] and its essential role in governing steady-state granulopoiesis is demonstrated by a 70–90% reduction in circulating neutrophils in G-CSF-deficient ($Csf3^{-/-}$) [8] and G-CSFR-deficient ($Csf3r^{-/-}$) [9] mice, respectively. However, the role of G-CSF during emergency granulopoiesis is less well established because of a shortage of conclusive experimental data. Although an older study revealed that G-CSF-deficient mice have a significantly attenuated granulopoietic response and increased lethality during

infection with *Listeria monocytogenes* compared with control mice [8,10], another study testing emergency granulopoiesis in the setting of systemic infection with *Candida albicans* did not detect differences in emergency granulopoiesis between *Candida*-infected G-CSF-deficient and control mice [11]. Data revealing that cytokines, most importantly G-CSF and macrophage colony-stimulating factor (M-CSF), not only provide survival and proliferative signals but are also able to directly instruct lineage choice in bipotent granulocyte–macrophage progenitors [12], together with the abundant literature on highly-elevated serum levels of myelopoietic cytokines during systemic infection [4▪,5], strongly suggest a pivotal role of growth facto rs during emergency granulopoiesis. However, the identity of the pathogen-sensing and cytokine-releasing cell type has remained enigmatic. A popular assumption was that monocytes and tissue-resident macrophages induce emergency granulopoiesis [5,6]. Indeed, monocytes and macrophages possess all of the theoretical functional requirements, and most importantly, express pattern recognition receptors and granulopoietic cytokines [such as G-CSF, granulocyte–macrophage colony-stimulating factor (GM-CSF), and M-CSF] upon stimulation with pathogens [13–16]. However, this notion has never been conclusively proven by stringent in-vivo experimentation. Only recently, the exact mechanism by which disseminated bacterial infection is sensed and translated into increased granulopoietic growth factor levels which, in turn, stimulate the switch from steady-state to emergency granulopoiesis has been clarified. We have generated bone marrow chimeric animals with a selective Toll-like receptor 4 (*Tlr4*) or myeloid differentiation primary response gene 88 (*Myd88*) deficiency in either hematopoietic or nonhematopoietic tissues [17,18▪▪]. Following stimulation with high doses of lipopolysaccharide (LPS) to mimic severe systemic Gram-negative bacterial infection, we could observe that while LPS-sensing by hematopoietic cells is dispensable for the induction of emergency granulopoiesis, *Tlr4* and *Myd88* expression within an irradiation-resistant nonhematopoietic cell type is absolutely required for this process. Moreover, the data also indicated that G-CSF is the primary cytokine driving LPS-induced emergency granulopoiesis [17]. To determine the exact identity of this nonhematopoietic cell type, we generated tissue-specific *Myd88* knockout mice using *Cre-loxP* recombination targeting several candidate nonhematopoietic cell types including *Nestin*$^+$ mesenchymal stromal cells (MSCs) [19,20], CXCL12-abundant reticular cells [21], and endothelial cells [22,23], all of which have been implicated in regulating several aspects of hematopoiesis [24▪▪,25]. The data unambiguously revealed that endothelial cells are the primary LPS-sensing cell type and the essential source of G-CSF in the course of LPS-induced emergency granulopoiesis (Fig. 1a). Although LPS-induced emergency granulopoiesis was completely dependent on endothelial cell-intrinsic *Myd88* expression, emergency granulopoiesis induced by *Escherichia coli* was still detectable in the setting of *Myd88* deficiency, however, at a significantly reduced magnitude and, most importantly, despite the lack of measurable G-CSF serum levels [18▪▪]. Consequently, alternative, G-CSF-independent pathways to initiate emergency granulopoiesis in the context of infection with live bacteria exist.

Interestingly, a recent study by Kwak *et al.* [26▪] revealed that mature myeloid cell-derived reactive oxygen species (ROS) externally regulate the proliferation of myeloid progenitors during emergency granulopoiesis. The authors found that stimulation with heat-inactivated Gram-negative bacterium *E. coli* leads to elevated levels of ROS within the bone marrow, and that ROS are released from Gr1$^+$ myeloid cells in a nicotinamide adenine dinucleotide phosphate oxidase-dependent manner. Of note, ROS-induced emergency granulopoiesis is apparently independent from G-CSF-induced emergency granulopoiesis and this finding provides a possible explanation for the G-CSF-independent emergency granulopoietic response observed by us in endothelial cell-specific *Myd88* knockout mice following infection with *E. coli*. Thus, ROS and G-CSF cooperate in regulating emergency granulopoiesis following infection with *E. coli*. Moreover, the study by Kwak *et al.* demonstrates that mature myeloid Gr1$^+$ cells regulate their own replenishment during emergency granulopoiesis via ROS-mediated stimulation of HSPCs (Fig. 1b).

In contrast to bacterial infection-induced emergency granulopoiesis, peripheral blood monocytosis is often observed during viral infection, suggesting initiation of an emergency monocytopoiesis program. De Bruin *et al.* [27] found that T cell-derived IFNγ during infection with lymphocytic choriomeningitis virus directly modulates lineage outcome in common myeloid progenitors and granulocyte-macrophage progenitors favoring monocyte generation at the expense of granulocyte production. On the molecular level, IFNγ suppresses G-CSF signal transduction via suppressor of cytokine signaling 3-dependent inhibition of signal transducer and activator of transcription 3 phosphorylation and concomitant induction of monocytopoiesis-stimulating transcription factors in myeloid progenitors [27].

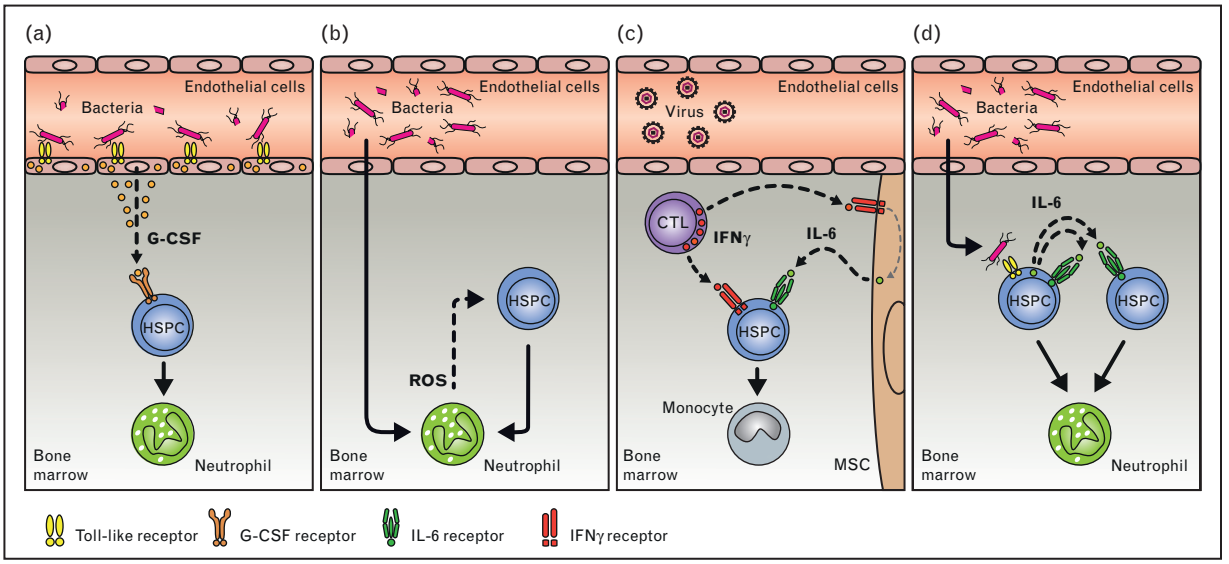

FIGURE 1. Pathways for sensing and translation of pathogen signals into emergency granulopoiesis and emergency monocytopoiesis. (a) Granulocyte colony-stimulating (G-CSF)-mediated emergency granulopoiesis. Bacteria and bacterial fragments (e.g., lipopolysaccharide) are sensed by Toll-like receptor-expressing endothelial cells and consequently release large amounts of G-CSF into the systemic circulation. Endothelial cell-derived G-CSF acts on G-CSF receptor-expressing hematopoietic stem and progenitor cells (HSPCs) and induce enhanced proliferation and neutrophil differentiation. (b) Reactive oxygen species (ROS)-mediated emergency granulopoiesis. During systemic bacterial infection, $Gr1^+$ myeloid cells including cells of the granulocytic lineage release ROS that stimulate increased proliferation and myeloid-biased differentiation of HSPCs. (c) IFNγ-mediated emergency monocytopoiesis. IFNγ released from cytotoxic T cells (CTLs) directly and indirectly promotes emergency monocytopoiesis. IFNγ directly acts on IFNγ receptor-expressing HSPCs thereby favoring monocytic differentiation at the expense of granulocytopoiesis. In addition, IFNγ stimulates IFNγ receptor-expressing bone marrow mesenchymal stromal cells (MSCs) to secrete IL-6, which together with other cytokines, stimulates monocytopoiesis from HSPCs expressing IL-6 receptor. (d) IL-6-mediated emergency granulopoiesis. Toll-like receptor-expressing HSPCs directly sense bacteria and bacterial fragments including lipopolysaccharide and lipoproteins. As a consequence, HSPCs secrete IL-6 which in conjunction with other cytokines contributes to emergency granulopoietic responses in an autocrine and paracrine manner.

Along similar lines, results from a recent study have not only shed new light on the role of IL-6 during emergency monocytopoiesis but also demonstrated unexpected cellular sources of this cytokine. IL-6 has been mainly implicated in the regulation of steady-state and emergency granulopoiesis [28,29]. It was shown that *Il6*$^{-/-}$ mice have an impaired neutrophil response after *C. albicans* infection [30], and mice simultaneously lacking G-CSF, GM-CSF and IL-6 harbor a more profound defect in granulopoiesis compared with mice with a combined absence of G-CSF and GM-CSF as demonstrated by in-vitro experimentation [31]. However, Schürch *et al.* [32▪▪] studied the hematopoietic response during systemic viral infection and found that IFNγ released by cytotoxic $CD8^+$ T cells activates the hematopoietic system and induces myeloid-biased differentiation favoring mainly monocytopoiesis. Moreover, using reciprocal bone marrow chimeric animals the authors could show that IFNγ does not directly act on HSPCs but stimulates an irradiation-resistant cell type to release IL-6, which in turn, mediates increased myelopoiesis with monocyte-biased differentiation. To identify the IL-6-secreting cell type, the authors isolated bone marrow MSCs, osteoblasts, and endothelial cells of wild-type and IFNγ-receptor knockout mice (*Ifngr*$^{-/-}$) and stimulated these cell populations *ex vivo* with IFNγ. Notably, IL-6 secretion was only observed in supernatants from wild-type MSC cultures. Altogether, these data corroborate a model (Fig. 1c), in which IFNγ released from $CD8^+$ T cells induces IL-6 secretion from bone marrow MSCs that stimulates enhanced myelopoiesis and monocytic differentiation [32▪▪].

DIRECT INITIATION OF DEMAND-ADAPTED MYELOPOIESIS-PATHOGEN SENSING BY HEMATOPOIETIC STEM AND PROGENITOR CELLS

A solid body of evidence demonstrates that HSPCs express TLRs [2,33,34] and that TLR stimulati on leads to proliferation and myeloid-biased

differentiation of mouse [33] and human HSPCs [35,36]. In conjunction with the well established migratory capacity of HSCs, regularly egressing and re-entering the bone marrow [37], a potential physiological function might be surveillance of tissues for the presence of invading pathogens followed by generation of myeloid offspring at the site of pathogen entry. Indeed, one study suggested such a mechanism, though under very special experimental settings, involving transplantation under the kidney capsule of ex-vivo prestimulated mouse HSPCS followed by prolonged in-vivo stimulation with LPS. Such treatment led to the detection clusters of myeloid cells within the kidney [38].

Additional support for a direct role of pathogen sensing by HSPCs has emerged recently. Zhao *et al.* employed a high-throughput, microfluidic-based platform to simultaneously measure 12 cytokines secreted from fluorescence-activated cell sorting-isolated HSPC populations upon ex-vivo stimulation with LPS and/or TLR2 agonist Pam3CSK4 [39▪▪]. Surprisingly, the authors could observe significant secretion of a variety of cytokines including IL-1β, IL-2, IL-4, IL-6, IL-12, IL-17A, IFNγ, TNFα, and GM-CSF at the single-cell level following TLR agonist stimulation. Notably, although the most immature immunophenotypically defined long-term HSCs were unresponsive to TLR agonist stimulation, cytokine release was most abundant in short-term HSCs and multipotent progenitors. Among the measured cytokines, IL-6 was most prominently detected both in terms of percentage of individual HSPCs expressing the cytokine as well as in the amount of secreted IL-6. The authors set out to test the biological relevance of HSPC-derived IL-6 but faced the experimental problem that it is impossible with current technology to abrogate IL-6 specifically in a defined HSPC population leaving other cell types unaffected. Therefore, the authors conceived an experimental setting, in which irradiated and consequently severe leukopenic hosts were transplanted with $Lin^{-}cKit^{+}$ cells from either wild-type or $Il6^{-/-}$ mice. Subsequently, recipient mice were stimulated with LPS. Although mice transplanted with $Il6^{-/-}$ cells showed some evidence for emergency granulopoiesis, the response was severely diminished compared with mice receiving wild-type cells [39▪▪]. Although these results suggest that HSPC-derived IL-6 is able to contribute to emergency granulopoiesis under very defined experimental conditions, that is, in an irradiated and severely leukopenic host, the relative contribution of other cellular sources of IL-6 such as bone marrow stromal cell populations in a more natural setting remains to be determined. Nevertheless, the study by Zhao *et al.* suggests that HSPCs might not only be targets for growth factor signals but, indeed, directly sense pathogen signals with the consequence of delivering cytokine signals in an autocrine and/or paracrine manner (Fig. 1d).

CONCLUSION

Adaptation of the blood-forming system to severe systemic, life-threatening infection, that is, one of the strongest naturally-occurring hematopoietic stressors, has been extensively studied over the past decades as outlined in this review. Recent findings have drawn a complex picture, in which various hematopoietic and nonhematopoietic cell types interact in an intricate manner to shape an appropriate hematopoietic response to a specific emergency situation (Fig. 1a–d). However, several important questions have remained unanswered and some limitations need to be taken into consideration when evaluating available data. Therefore, we suggest particularly addressing the following issues when conceiving new experiments to further elucidate our understanding of demand-adapted myelopoiesis.

First, it will be important to use appropriate types of pathogens given the fact that previous results suggested pathogen-specific differences in emergency hematopoietic responses. For instance, while the studies using pathogens such as intracellular bacterium *L. monocytogenes* and yeast *C. albicans* certainly yielded valuable results, both pathogens are less frequent in causing hematopoietic emergency responses compared with common pathogenic bacteria such as *Staphylococci* and *Enterobacteriacae*, and potential studies using the latter might yield different results. Second, we suggest to evaluate and report findings on proliferation of HSPCs in the bone marrow and evidence for enhanced cell turnover together with results of peripheral blood counts. Isolated quantitative differences in peripheral blood might be solely a consequence of mobilization of preformed myeloid cells from storage pools such as the bone marrow. Similarly, a change in numbers of myeloid progenitor cells in the bone marrow without changes in peripheral blood counts or indications for accelerated cell turnover as measured by, for instance, 5-bromo-2′-deoxyuridine incorporation, would also be insufficient. Third, it will be interesting to address the question of where pathogen sensing and production of myelopoiesis-stimulating cytokines primarily takes place, that is, inside or outside the bone marrow, and whether both possibilities might differ with respect to excess of infection and the functional outcome.

In summary, further studies are warranted to elucidate the relative contribution of the various above-described mechanisms operating to regulate the switch from steady-state to demand-adapted myelopoiesis with the overall goal to ensure proper context- and pathogen-dependent functionality of the hematopoietic system. In addition, research on this holds the promise to devise new therapeutic strategies to treat human infectious and hematologic diseases.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

1. Kondo M, Wagers AJ, Manz MG, *et al.* Biology of hematopoietic stem cells and progenitors: implications for clinical application. Annu Rev Immunol 2003; 21:759–806.
2. Takizawa H, Boettcher S, Manz MG. Demand-adapted regulation of early hematopoiesis in infection and inflammation. Blood 2012; 119:2991–3002.
3. Kolaczkowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. Nat Rev Immunol 2013; 13:159–175.
4. ■ Manz MG, Boettcher S. Emergency granulopoiesis. Nat Rev Immunol 2014; 14:302–314.

A comprehensive review on cellular and molecular aspects of emergency granulopoiesis.

5. Kaushansky K. Lineage-specific hematopoietic growth factors. N Engl J Med 2006; 354:2034–2045.
6. Hamilton JA. Colony-stimulating factors in inflammation and autoimmunity. Nat Rev Immunol 2008; 8:533–544.
7. Panopoulos AD, Watowich SS. Granulocyte colony-stimulating factor: molecular mechanisms of action during steady state and 'emergency' hematopoiesis. Cytokine 2008; 42:277–288.
8. Lieschke GJ, Grail D, Hodgson G, *et al.* Mice lacking granulocyte colony-stimulating factor have chronic neutropenia, granulocyte and macrophage progenitor cell deficiency, and impaired neutrophil mobilization. Blood 1994; 84:1737–1746.
9. Liu F, Wu HY, Wesselschmidt R, *et al.* Impaired production and increased apoptosis of neutrophils in granulocyte colony-stimulating factor receptor-deficient mice. Immunity 1996; 5:491–501.
10. Zhan Y, Lieschke GJ, Grail D, *et al.* Essential roles for granulocyte-macrophage colony-stimulating factor (GM-CSF) and G-CSF in the sustained hematopoietic response of Listeria monocytogenes-infected mice. Blood 1998; 91:863–869.
11. Basu S, Hodgson G, Zhang HH, *et al.* 'Emergency' granulopoiesis in G-CSF-deficient mice in response to Candida albicans infection. Blood 2000; 95:3725–3733.
12. Rieger MA, Hoppe PS, Smejkal BM, *et al.* Hematopoietic cytokines can instruct lineage choice. Science 2009; 325:217–218.
13. He RL, Zhou J, Hanson CZ, *et al.* Serum amyloid A induces G-CSF expression and neutrophilia via Toll-like receptor 2. Blood 2009; 113:429–437.
14. Vellenga E, Rambaldi A, Ernst TJ, *et al.* Independent regulation of M-CSF and G-CSF gene expression in human monocytes. Blood 1988; 71:1529–1532.
15. de Waal Malefyt R, Abrams J, Bennett B, *et al.* Interleukin 10(IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. J Exp Med 1991; 174:1209–1220.
16. Nishizawa M, Nagata S. Regulatory elements responsible for inducible expression of the granulocyte colony-stimulating factor gene in macrophages. Mol Cell Biol 1990; 10:2002–2011.
17. Boettcher S, Ziegler P, Schmid MA, *et al.* Cutting edge: LPS-induced emergency myelopoiesis depends on TLR4-expressing nonhematopoietic cells. J Immunol 2012; 188:5824–5828.
18. ■■ Boettcher S, Gerosa RC, Radpour R, *et al.* Endothelial cells translate pathogen signals into G-CSF-driven emergency granulopoiesis. Blood 2014; 124:1393–1403.

Together with reference [17] seminal studies demonstrating that endothelial cells are critical regulators during systemic bacterial infection by translating LPS and *E. coli* signals into enhanced G-CSF secretion and subsequent initiation of emergency granulopoiesis.

19. Mèndez-Ferrer S, Michurina TV, Ferraro F, *et al.* Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. Nature 2010; 466:829–834.
20. Shi C, Jia T, Mendez-Ferrer S, *et al.* Bone marrow mesenchymal stem and progenitor cells induce monocyte emigration in response to circulating toll-like receptor ligands. Immunity 2011; 34:590–601.
21. Sugiyama T, Kohara H, Noda M, Nagasawa T. Maintenance of the hematopoietic stem cell pool by CXCL12-CXCR4 chemokine signaling in bone marrow stromal cell niches. Immunity 2006; 25:977–988.
22. Ding L, Saunders TL, Enikolopov G, Morrison SJ. Endothelial and perivascular cells maintain haematopoietic stem cells. Nature 2012; 481:457–462.
23. Ding L, Morrison SJ. Haematopoietic stem cells and early lymphoid progenitors occupy distinct bone marrow niches. Nature 2013; 495:231–235.
24. ■■ Morrison SJ, Scadden DT. The bone marrow niche for haematopoietic stem cells. Nature 2014; 505:327–334.

An excellent review on the various cell types of the bone marrow niche with a focus on their function regarding maintenance of hematopoietic stem cells.

25. Mendelson A, Frenette PS. Hematopoietic stem cell niche maintenance during homeostasis and regeneration. Nat Med 2014; 20:833–846.
26. ■ Kwak H-J, Liu P, Bajrami B, *et al.* Myeloid cell-derived reactive oxygen species externally regulate the proliferation of myeloid progenitors in emergency granulopoiesis. Immunity 2015; 42:159–171.

The first study to demonstrate that myeloid cell-derived ROS promotes myeloid progenitor proliferation during emergency granulopoiesis.

27. de Bruin AM, Libregts SF, Valkhof M, *et al.* IFNγ induces monopoiesis and inhibits neutrophil development during inflammation. Blood 2012; 119:1543–1554.
28. Kopf M, Baumann H, Freer G, *et al.* Impaired immune and acute-phase responses in interleukin-6-deficient mice. Nature 1994; 368:339–342.
29. Bernad A, Kopf M, Kulbacki R, *et al.* Interleukin-6 is required in vivo for the regulation of stem cells and committed progenitors of the hematopoietic system. Immunity 1994; 1:725–731.
30. Romani L, Mencacci A, Cenci E, *et al.* Impaired neutrophil response and CD4+ T helper cell 1 development in interleukin 6-deficient mice infected with Candida albicans. J Exp Med 1996; 183:1345–1355.
31. Walker F, Zhang H-H, Matthews V, *et al.* IL6/sIL6R complex contributes to emergency granulopoietic responses in G-CSF- and GM-CSF-deficient mice. Blood 2008; 111:3978–3985.
32. ■■ Schürch CM, Riether C, Ochsenbein AF. Cytotoxic CD8+ T cells stimulate hematopoietic progenitors by promoting cytokine release from bone marrow mesenchymal stromal cells. Cell Stem Cell 2014; 14:460–472.

A very interesting study showing that bone marrow stromal cells respond to CTL-derived IFNγ with release of IL-6 that promotes myelopoiesis and monocyte differentiation.

33. Nagai Y, Garrett KP, Ohta S, *et al.* Toll-like receptors on hematopoietic progenitor cells stimulate innate immune system replenishment. Immunity 2006; 24:801–812.
34. Schmid MA, Takizawa H, Baumjohann DR, *et al.* Bone marrow dendritic cell progenitors sense pathogens via Toll-like receptors and subsequently migrate to inflamed lymph nodes. Blood 2011; 118:4829–4840.
35. De Luca K, Frances-Duvert V, Asensio M-J, *et al.* The TLR1/2 agonist PAM(3)CSK(4) instructs commitment of human hematopoietic stem cells to a myeloid cell fate. Leukemia 2009; 23:2063–2074.
36. Sioud M, Fløisand Y, Forfang L, Lund-Johansen F. Signaling through toll-like receptor 7/8 induces the differentiation of human bone marrow CD34+ progenitor cells along the myeloid lineage. J Mol Biol 2006; 364:945–954.
37. Wright DE, Wagers AJ, Gulati AP, *et al.* Physiological migration of hematopoietic stem and progenitor cells. Science 2001; 294:1933–1936.
38. Massberg S, Schaerli P, Knezevic-Maramica I, *et al.* Immunosurveillance by hematopoietic progenitor cells trafficking through blood, lymph, and peripheral tissues. Cell 2007; 131:994–1008.
39. ■■ Zhao JL, Ma C, O'Connell RM, *et al.* Conversion of danger signals into cytokine signals by hematopoietic stem and progenitor cells for regulation of stress-induced hematopoiesis. Cell Stem Cell 2014; 14:445–459.

The study reports the interesting finding that HSPCs respond to direct TLR agonist stimulation with secretion of considerable amounts of a variety inflammatory cytokines and growth factors, which probably promote demand-adapted hematopoiesis.